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(54) Title: MICROORGANISM PRODUCING 5'XANTHYLIC ACID

(57) Abstract: The invention relates to *Corynebacterium ammoniagenes* CJXOL 0201 KCCM 10447 producing 5'-xanthylic acid. More specifically, the invention relates to *Corynebacterium ammoniagenes*CJXOL 0201 KCCM 10447 which is a mutant strain of *Corynebacterium ammoniagenes* KCCM 10340 having a resistance to oligomycin. In order to obtain mutant strain having enhanced respiratory activity, the present invention adopted *Corynebacterium ammoniagenes* KCCM 10340 as parent strain and treated it with UV radiation and mutation derivatives such as N-methyl-N'-nitro-n-nitrosoguanidine(NTG) according to ordinary procedure. Therefore, *Corynebacterium ammoniagenes* CJXOL 0201 KCCM 10447 of the present invention makes it possible to increase ATP reproducing activity for same period of fermentation and can accumulate 5'xanthylic acid in culture medium at a high yield and concentration rate.

MICROORGANISM PRODUCING 5'-XANTHYLIC ACID

Technical Field

The invention relates to a microorganism producing 5'-xanthylic acid. More particularly, the invention relates to a mutant strain of *Corynebacterium ammoniagenes* KCCM 10340 having a resistance to oligomycin which inhibits ATP synthase activity and oxidative phosphorylation process, in order to enhance respiratory activity, making it possible to enhance ATP reproducing activity for same period of fermentation and accumulate 5'-xanthylic acid in culture medium at a high yield and high concentration rate.

Background Art

5'-xanthylic acid is an intermediate in the nucleic acid biosynthesis process, which is physiologically important in the body of animals and plants, used in food, medical supplies and other various field. The invention relates to a mutant strain having a resistance to oligomycin, obtained from a known strain *Corynebacterium ammoniagenes* KCCM 10340, producing 5'-xanthylic acid at a high yield and high concentration rate by a direct fermentation method.

5'-xanthylic acid is an intermediary product of purine nucleotide biosynthesis process and important material for producing 5'-guanylic acid. A widely used method to produce 5'-guanylic acid having fineness and high quality is microorganism fermentation method which produces 5'-xanthylic acid first and converts it into 5'-guanylic acid enzymatically, therefore, to produce 5'-guanylic acid, corresponding amount of 5'-xanthylic acid is necessary. Conventional methods to produce 5'-xanthylic acid are chemosynthesis, deaminization of 5'-guanylic acid which is produced as a result of decomposition of ribonucleic acid in yeast, a fermentation method to add xanthine as precursor material in

fermenting medium, a fermentation method to use a mutant strain of microorganism, a method to add antibiotic material (JP 1477/42 and JP 20390/44), a method to add surfactant (JP 3825/42 and JP 3838/42) and so on. Among these, a direct fermentation method of 5'-xanthylic acid by a mutant strain of microorganism is quite advantageous in terms of industrial aspect. Thus, we inventors developed a mutant strain with increased productivity of 5'-xanthylic acid, by modifying the existing character of *Corynebacterium ammoniagenes* KCCM 10340 into the character of producing 5'-xanthylic acid at a large yield rate.

Most microorganisms reach to the condition that the volume doesn't increase any more when keep on culturing under the constant condition, and especially the concentration of microorganism producing primary metabolite, a growth-dependent product, doesn't increase any more. It is mainly caused by limited supply of dissolved oxygen. Method of enhancing aeration and agitation condition, for removal of the limited supply of dissolved oxygen, is used, but there is technical and economical limit in actual production method. To overcome the limit and increase yield rate and concentration of 5'-xanthylic acid by enhancing the volume of microorganism and various physiological activity, under the limited supply of dissolved oxygen, we inventors thought that the method of enhancing respiratory activity and ATP reproducing activity of microorganism under the same dissolved oxygen would be useful. Thus, the inventors investigated microorganism strains having a resistance to various respiratory inhibitors, and found out that a mutant strain having a resistance to oligomycin is most effective among these and can produce 5'-xanthylic acid at a high yield and high concentration rate by a direct fermentation method, and accomplished in this invention.

Disclosure of the Invention

The invention relates to *Corynebacterium ammoniagenes* CJXOL 0201 (KCCM-10447) which is a mutant strain of *Corynebacterium ammoniagenes* KCCM 10340, producing 5'-xanthylic acid. The CJXOL 0201 is obtained by treating *Corynebacterium ammoniagenes* KCCM 10340 with UV radiation and mutation derivatives such as N-methy-N'-nitro-n-nitrosoguanidine(NTG) according to ordinary procedure, and selecting a mutant strain among these which can grow in the culture medium (glucose 20g/L, potassium phosphate monobasic 1g/L, potassium phosphate dibasic 1g/L, urea 2g/L, ammonium sulfate 3g/L, magnesium sulfate 1g/L, calcium chloride 100mg/L, ferrous sulfate 20mg/L, manganese sulfate 10mg/L, zinc sulfate 10mg/L, biotin 30μg/L, thiamine hydrochloride 0.1mg/L, copper sulfate 0.8mg/L, adenine 20mg/L, guanine 20mg/L, pH 7.2) which different concentration levels of oligomycin (1,2,5,10,20,50mg/L) is added into. In the procedure, 0~50mg/L oligomycin was added into the medium and there showed a resistance up to 20mg/L oligomycin but no growth was observed at the concentration level above 20mg/L. A strain which can grow in 20mg/L oligomycin was separated, named CJXOL 0201, and it was deposited under Budapest Treaty to the Korean Culture Center of Microorganisms on November 21, 2002 with accession Number KCCM 10447.

The biochemical characteristic of the novel mutant strain CJXOL 0201 of the invention is shown in the following Table 1. According to the Table 1, the microorganism of the invention can grow in the medium which 10mg/L oligomycin was added into. The medium was fermented at 30℃ for 5 days.

Table 1

Strain	Oligomycin Concentraion (mg/L)						
	0	5	10	20	30	40	50

KCCM 10340	+++	++	+	-	-	-	-
CJXOL 0201	+++	+++	++	++	+	-	-

+ : growth, - : no growth

BEST MODE FOR CARRYING OUT THE INVENTION

Example 1

Used strains: *Corynebacterium ammoniagenes* KCCM 10340, *Corynebacterium ammoniagenes* CJXOL 0201

Seed medium: glucose 30g/L, peptone 15g/L, yeast extract 15g/L, sodium chloride 2.5g/L, urea 3g/L, adenine 150mg/L, guanine 150mg/L, pH 7.2

Fermentation medium: (1) A medium: glucose 60g/L, magnesium sulfate 10g/L, ferrous sulfate 20mg/L, zinc sulfate 10mg/L, manganese sulfate 10mg/L, adenine 30mg/L, guanine 30mg/L, biotin 100μg/L, copper sulfate 1mg/L, thiamine hydrochloride 5mg/L, calcium chloride 10mg/L, pH 7.2

(2) B medium: potassium phosphate monobasic 10g/L, potassium phosphate dibasic 10g/L, urea 7g/L, ammonium sulfate 5g/L

Fermentation method: 5mL of the seed medium was poured into a test tube having diameter of 18mm and sterilized under pressure according to the common methods. After the sterilization, *Corynebacterium ammoniagenes* KCCM 10340 and *Corynebacterium ammoniagenes* CJXOL 0201 were seeded into respectively and it was cultured with shaking at 180rpm, 30℃ for 18 hours. The resultant was used as seed culture. Then, as fermentation medium, A medium and B medium

were sterilized separately under pressure according to the common methods and 29mL of A medium and 10mL of B medium were respectively poured into sterilized 500mL-Erlenmeyer flask for shaking and 1mL of the above-mentioned seed culture was seeded into and fermented at 200rpm, 30℃ for 90 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10340 was 23.0g/L and the amount in CJXOL 0201 was 26.5g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Example 2

Used strains: same as example 1.

Primary seed medium: same as the seed medium of example 1.

Secondary seed medium: glucose 60g/L, potassium phosphate monobasic 2g/L, potassium phosphate dibasic 2g/L, magnesium sulfate 1g/L, ferrous sulfate 22mg/L, zinc sulfate 15mg/L, manganese sulfate 10mg/L, copper sulfate 1mg/L, calcium chloride 100mg/L, biotin 150μg/L, adenine 150mg/L, guanine 150mg/L, thiamine hydrochloride 5mg/L, antifoaming agent 0.6mL/L, pH 7.2

Fermentation medium: glucose 151g/L, phosphoric acid 32g/L, potassium hydroxide 25g/L, adenine 198mg/L, guanine 119mg/L, ferrous sulfate 60mg/L, zinc sulfate 42mg/L, manganese sulfate 15mg/L, copper sulfate 2.4mg/L, alaniate 22mg/L, NCA 7.5mg/L, biotin 0.4mg/L, magnesium sulfate 15g/L, cystinate 30mg/L, histidinate 30mg/L, calcium chloride 149mg/L, thiamine hydrochloride 15mg/L, antifoaming agent 0.7mL/L, CSL 27mL/L, tuna extract 6g/L, pH 7.3

Primary seed culture: 50mL of the primary seed medium was poured into 500mL-Erlenmeyer flask for shaking and sterilized under pressure at 121℃ for 20 minutes. After cooling, *Corynebacterium ammoniagenes* KCCM 10340 and *Corynebacterium ammoniagenes* CJXOL 0201 were seeded into respectively and it was cultured with shaking at 180rpm, 30℃ for 24 hours.

Secondary seed culture: The secondary seed medium was poured into 5L-experimental fermentation baths (2L each) and sterilized under pressure at 121℃ for 10 minutes. After cooling, 50mL of the above primary seed culture was seeded and cultured with the air supply of 0.5vvm, at 900rpm, 31℃, for 24 hours. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting by ammonia solution.

Fermentation method: The fermentation medium was poured into 30L-experimental fermentation baths (8L each) and sterilized under pressure at 121℃ for 20 minutes. After cooling, the above secondary seed culture was seeded into (1.5L each) and cultured with the air supply of 1vvm, at 400rpm, 33℃. Whenever the residual sugar level drops below 1% during the culturing process, sterilized glucose was supplied and the total sugar level in the fermentation medium was kept at 30%. During the culturing process, the pH level of the medium was kept at 7.3 with adjusting by ammonia solution and the process took 90 hours. After the fermentation was completed, the amount of accumulation of 5'-xanthylic acid in the medium showed that the amount in KCCM 10340 was 137.2g/L and the amount in CJXOL 0201 was 148.4g/L. (The concentration of accumulated 5'-xanthylic acid is given by 5'-sodium xanthate·$7H_2O$.)

Industrial Applicability

The invention adopted *Corynebacterium ammoniagenes* KCCM 10340 as parent strain and treated it UV radiation or mutation derivatives such as N-methy-N'-nitro-n-nitrosoguanidine (NTG) according to ordinary procedure. The KCCM 10340 strain has a resistance to osmotic pressure, caused by high concentration of 5'-xanthylic acid accumulated during culturing process, high concentration of glucose and various carbon source added into culture medium, which results in the high osmotic pressure outside bacterial body, inhibition of normal physiological activity of 5'-xanthylic acid-producing cell and decrease of 5'-xanthylic acid production. In order to obtain a strain having enhanced character of osmotic pressure resistance and enhanced respiratory activity, the invention modified *Corynebacterium ammoniagenes* KCCM 10340 and selected a mutant strain having a resistance to oligomycin which inhibits ATP synthase activity and oxidative phosphorylation process, and makes it possible to enhance ATP reproducing activity and to accumulate 5'-xanthylic acid in culture medium at a high yield and high concentration rate for same period of fermentation.

WHAT IS CLAIMED IS:

1. *Corynebacterium ammoniagenes* CJXOL 0201 (Accession Number: KCCM 10447) having a resistance to oligomycin and producing 5'-xanthylic acid.

2. A method of producing 5'-xanthylic acid characterized by using *Corynebacterium ammoniagenes* CJXOL 0201 (Accession Number: KCCM 10447) of claim 1.

Applicant's or agent's file reference	International application No.
YL03017PCT	PCT/KR2003/002703

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 3, line 18-19

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

Korean Culture Center of Microorganisms(KCCM)

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D
Hongje 1-dong, Seodaemun-gu
120-091 Seoul
Republic of Korea

Date of deposit	Accession Number
November 21, 2002	KCCM-10447

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/KR2003/002703

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC7 C12N 1/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
CA, Delphion, PubMed, KIPASS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 12-295996 A (TAKANOYUTAKA) 24 OCTOBER 2000 See the whole documents	1-2
A	EP 0350970 A (KYOWA HAKKO KOGYO CO., LTD.) 17 JANUARY 1990 See the abstract	1-2
A	KR 02-57470 A (CJ CORP.) 11 JULY 2002 See the abstract	1-2
A	KR 01-89980 A (CJ CORP.) 01 OCTOBER 2001 See the abstract	1-2
A	JP 59-078687 A (KYOWA HAKO KOGYO Co., LTD) See the abstract	1-2

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 MARCH 2004 (30.03.2004)	30 MARCH 2004 (30.03.2004)

Name and mailing address of the ISA/KR	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2003/002703

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP0350970A1	17.01.1990	CA1215338A1	16.12.1986
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